Integration of a Diagnostic Into an Advanced Wound Care Dressing

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Expressive Constructs, Inc

Introduction

We have developed a simple, [illegible] safe, and rapid diagnostic sensor that detects the [illegible] of a high [illegible]den of microorganisms in wounds before there are any visible clinical signs. The sensor measures proteases produced by bacterial pathogens but does not cross react with human fluids or human [illegible] such as MMPs and human neutrophil elastase. The dressing sensor is simple [illegible] to use a food grade [illegible] collect on the surface of the dressing, inexpensive enough that the components cost less than ten cents, generally regarded as safe (GRAS) to go on your skin or in a [illegible] to change color in 10 minutes to an hour.

The diagnostic dressing [illegible] is incorporated into a polyurethane foam wound dressing that could be [illegible] on a patient in order to continuously monitor for an infection. Clinical [illegible] to measure the performance of the dressing sensor with [illegible] wounds with the long term of objectives of [illegible] on the [illegible]. The [illegible] us to [illegible]ture and use a [illegible] sensor diagnostic [illegible] to [illegible] monitor the bacterial [illegible] [illegible] allow clinicians to treat a wound infection early, [illegible] wound healing and increasing the appropriate use of antibiotics and/or [illegible] clinical therapies.

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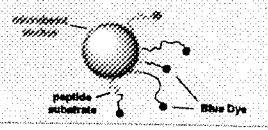
6. Cutting KF, Ellis [illegible], Yeboah B, Rodkin A, Dunn B, Sanders MC. "Rapid Bioassessment of Protease Activity [illegible] Wounds: A Diagnostic for Total Bioburden" submitted Journal of Wound Repair and Regeneration, March 2009.

7. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg Clin North Am 1997;77(3):637-50.7. Lazarus GS. Wound and Soft Tissue Cultures. In:

8. Isenberg HD, editor. [illegible] Handbook. Washington DC: ASM Press; 2004:11.1.1-11.1.18. Bowler PG. The [illegible] aerobic microbiology of wounds: a review. Wounds 1998;10:170-8.

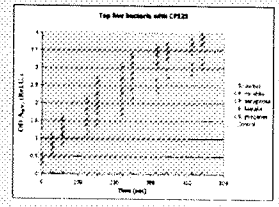
ExpressDetect® Assay Background

The assay detects bacterial [illegible], by measuring the proteolytic enzymes (proteases) they release into the [illegible]. Proteases are [illegible] factors [illegible] protect [illegible] bacteria during infection [illegible] matrix proteins, immune defense proteins and antimicrobial peptides. [illegible] are specifically measured by incorporating a proprietary peptide [illegible] rapid assay format.



Validation of Proteases as Markers for Wound Bacteria

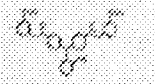
In vitro [illegible] of bacterial [illegible] using biotin-[illegible]idine tagged CPZ2 [illegible]. Shown are [illegible] efficient [illegible] of bacterial proteases [illegible] growth medium from cultures of *S. aureus*, *E. faecalis*, *S. progenes*, *P. mirabilis*, or *E. coli* adjusted to 10^6 CFU/ml or a control sample (blue). The media from all the bacterial species up to 24 hours of [illegible] and gave the [illegible] signal. Subsequent assays to simplify the assay. These samples were [illegible] on [illegible] coated with CPZ2 beads with a [illegible] cleavage time. The [illegible] removed by centrifugation and free dual tag was measured in an [illegible] using [illegible] and [illegible] (TA-HRP. Signal was measured by monitoring the [illegible] (450 nm) after addition of TMB substrate.



Food Grade Dyes as Diagnostic Sensors

Staining of CPZ2 Beads with Blue Dye #1

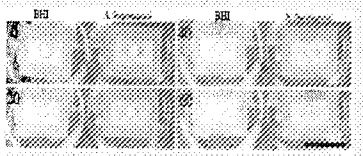
We have [illegible] with Molecular Probes, a Division of Invitrogen (Eugene Oregon), to [illegible] FD&C blue dye 1 (*orange juice*). Functionalized with [illegible], the chemical structure of this food grade dye is [illegible], which is [illegible] (*orange juice*) [illegible] (*Lactobacillus casei*), [illegible] (*S. pyogenes*), [illegible] (*S. epidermidis*), [illegible] (*Staphylococcus aureus*), [illegible] (*Escherichia coli*), [illegible] (*Pseudomonas aeruginosa*) (ATCC 45-9)), as shown below. We have [illegible] the [illegible] release of blue dye #1 by [illegible] using a [illegible] ratio of 1:5 in [illegible] buffer pH 7.5, for 3 hours at room temperature. The [illegible] peptide will be [illegible] from the [illegible] dye using a 4% solution and then the dye peptide conjugate will be [illegible] to [illegible] the bound [illegible] quantitatively.



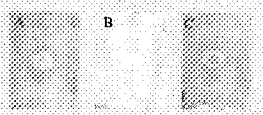
Chemical structure of Brilliant Blue FCF

Testing of Dressing Sensor with Whole Blood

ECI [illegible] has a protease [illegible] [illegible] cancer cells. The dressing sensor [illegible] the common wound pathogens as shown in the previous figure. In addition to a [illegible] which is an emerging pathogen in military patients that have blast induced trauma.



Dressing Sensor Test Test as with *S. faecalis*. In the presence of culture [illegible] for 0, 20, 40, or 60 minutes. Blue color indicates the presence of the protease. Scale Bar = 4 cm.

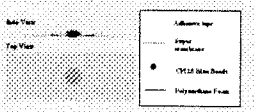


Testing of Supernatants [illegible] with whole blood. Uninfected whole blood diluted 1/2 with PBS (A) or PBS alone (B) had no cross reactivity with the sensor. The overnight cultures of *Staphylococcus epidermidis* ATCC 35984 (Blue color on the surface of the dressing sensor that is clearly visible in (A) sensor.

Experimental Conclusions

1. **Proteases appear to be valid markers for wound bacteria**
2. **The ExpressDetect® assay can detect *bacterial* proteases produced at the threshold value of 10^6 *CFU/ml in vitro***
3. **Bacterial protease activity in samples *from* chronic wounds correlates *with* to the concentration of *bacteria (bioburden)* found in *the wound***

Integration of Assay into a Dressing Sensor



Creating [illegible] prototype. The [illegible] dressing [illegible] of a [illegible] foam [illegible] layer, the CPZ2 [illegible] bead [illegible] and a top [illegible] layer (spec 450) to [illegible] for use in the presence of a [illegible] bio-burden of > 10^6 CFU/ml

Clinical Utility of Diagnostic Dressing Test

* A dressing [illegible] would be useful in chronic wounds with a [illegible] infection status:
  * [illegible] with or without clinical signs of infection
  * Will [illegible] in making a correct and immediate diagnosis of local wound infection
  * [illegible] treatment decision to reduce the bacterial level with a topical [illegible]
  * reduce both [illegible] negative and false positive [illegible]
    * decrease the inappropriate use of antibiotics in [illegible] patients
    * [illegible] appropriate [illegible] treatment to [illegible] personalized
* Not be an additive step in the [illegible] treatment regimen
  * eliminate [illegible] a swab or biopsy where infection is suspected to the wound bed
  * topical [illegible] agents are broad-spectrum [illegible]
  * Test result will be [illegible] even without the ability to determine the infecting organisms
* Test wounds being prepared for costly treatment or procedural options
  * growth factors, skin grafts, or bioengineered skin